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MINI REVIEW

Mice deficient in small leucine-rich proteoglycans: novel *in vivo* models for osteoporosis, osteoarthritis, Ehlers-Danlos syndrome, muscular dystrophy, and corneal diseases

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Small leucine-rich proteoglycans (SLRPs) are extracellular molecules that bind to TGFβs and collagens and other matrix molecules. *In vitro*, SLRPs were shown to regulate collagen fibrillogenesis, a process essential in development, tissue repair, and metastasis. To better understand their functions *in vivo*, mice deficient in one or two of the four most prominent and widely expressed SLRPs (biglycan, decorin, fibromodulin, and lumican) were recently generated. All four SLRP deficiencies result in the formation of abnormal collagen fibrils. Taken together, the collagen phenotypes demonstrate a cooperative, sequential, timely orchestrated action of the SLRPs that altogether shape the architecture and mechanical properties of the collagen matrix. In addition, SLRP-deficient mice develop a wide array of diseases (osteoporosis, osteoarthritis, muscular dystrophy, Ehlers-Danlos syndrome, and corneal diseases), most of them resulting primarily from an abnormal collagen fibrillogenesis. The development of these diseases by SLRP-deficient mice suggests that mutations in SLRPs may be part of undiagnosed predisposing genetic factors for these diseases. Although the distinct phenotypes developed by the different singly deficient mice point to distinct *in vivo* function for each SLRP, the analysis of the double-deficient mice also demonstrates the existence of rescuing/compensation mechanisms, indicating some functional overlap within the SLRP family.

Key words: biglycan/collagen/decorin/fibromodulin/lumican

Structure of the small leucine-rich proteoglycans

Small leucine-rich proteoglycans (SLRPs) belong to the leucine-rich repeat (LRR) superfamily of proteins (Hocking *et al.*, 1998). Members of the LRR superfamily may contain up to 38 LRRs. The LRR domain is 20 to 29 amino acids long with asparagine (N) and leucine (L) residues in conserved positions (Hocking *et al.*, 1998; Iozzo, 1999). The LRR is a structural motif used in diverse molecular recognition processes, including cell adhesion, signal transduction, DNA repair, and RNA processing (Kobe and Deisenhofer, 1994). It consists of a β-sheet (LxxLxLxxNxL) parallel to an α-helix (xaxx±a±±±±a±±x) where a is an aliphatic amino acid, x is any amino acid, and ± is frequently a gap.

Within the LRR superfamily, the SLRPs form a specific subgroup (Iozzo and Murdock, 1996). The SLRP family is uniquely characterized by cysteine-rich clusters, which form disulfide bonds and flank the central LRRs (see Figure 1 for example of SLRP structure). There are four similarly spaced cysteine residues in the amino terminal end and two cysteine residues in the carboxy terminal end. The SLRPs are called small to distinguish them from other much larger proteoglycans, like versican or aggrecan. The SLRP protein cores have a mass of approximately 40 kDa, compared with more than 200 kDa for versican and aggrecan (Iozzo, 1998).

The SLRP family is rapidly growing and includes today 13 members (Table I). So far, all SLRPs are extracellular except for one member (nyctalopin), which is a glycosylphosphatidyl-inositol-anchored protein (Bech-Hansen *et al.*, 2000; Pusch *et al.*, 2000). Most (but not all) SLRPs are proteoglycans (at least in some tissues and at some developmental stage or age). At least one member, asporin, is placed in the SLRP family based on gene sequence homology, even though it does not contain glycosaminoglycan chains. Molecular modeling and electron microscopy suggest that SLRPs are nonglobular, horseshoe-shaped, solenoid-like molecules (Figure 2) (Scott, 1996; Weber *et al.*, 1996). The concave surface of the horseshoe-shaped SLRPs is formed by the LRR's β-sheets, whereas the LRR's α-helices and the SLRP's diverse carbohydrate moieties flank the horseshoe convex surface.

Based on the genomic organization, structure of the amino terminal cysteine cluster, and similarity of the amino acid sequences, most SLRPs can be grouped into three classes (Table I) (Iozzo, 1999; Lorenzo *et al.*, 2001). Class I consists of decorin (DCN) (Ameye *et al.*, 2002b; Danielson *et al.*, 1993; Krusius and Ruoslahti, 1986; Scholzen *et al.*, 1994; Vetter *et al.*, 1993), biglycan (BGN) (Fisher *et al.*, 1989; Wegrowski *et al.*, 1995; Young and Chen, 2002), and asporin/PLAP (Lorenzo *et al.*, 2001; Henry *et al.*, 2001; Yamada *et al.*, 2001). Class I SLRPs are secreted with a propeptide that in some cases can be cleaved by bone morphogenetic protein-1 under age and tissue-specific conditions (Scott *et al.*, 2000). They contain 10 LRRs and a unique N-terminal cysteine sequence (CX_3CXCX_6C) and are encoded by eight exons (see Figure 1 for BGN structure). Asporin is not a proteoglycan, but DCN and BGN are. They carry one and two chondroitin or dermatan sulfate chains,

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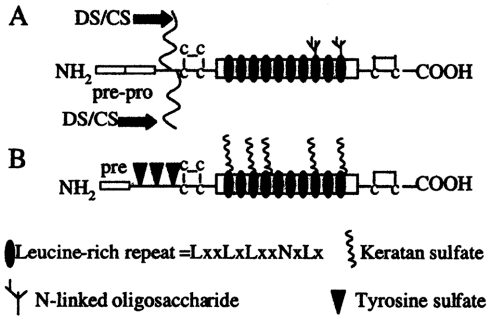

Fig. 1. Biglycan (A) and fibromodulin (B) structure. DS/CS: dermatan/chondroitin sulfate chains, pre: prepeptide, pre-pro: pre-propeptide.

Table I. Small leucine-rich proteoglycan classification

	SLRP	Gene and protein structure, and amino terminal cysteine cluster
Class I	asporin	8 exons
	biglycan	10 LRR
	decorin	CX_3CXCX_6C
Class II	fibromodulin	3 exons
	lumican	10 LRR
	keratocan	CX_3CXCX_9C
	PRELP	
	osteoadherin/osteomodulin	
Class III	epiphican/PG-Lb/DSPG3	7 exons
	mimican/osteoglycin	6 LRR
	opticin/oculoglycan	CX_2CXCX_6C
Others	chondroadherin	3 exons
	nyctalopin	11 LRR
		CX_3CXCX_8C or CX_3CXCX_6C

Classes of SLRPs were developed based on gene structure and DNA and protein homology. The first column lists different classes of SLRPs. The second column shows the members of the SLRP family belonging to each class. The third column lists the number of exons in the gene, the number of LLRs in the protein in each class, and the sequence of the amino terminal cysteine cluster (where C represents a cysteine and X any amino acid).

respectively. The attachment of chondroitin versus dermatan sulfate chains is tissue-specific (Hocking *et al.*, 1998).

Class II consists of fibromodulin (FM) (Antonsson *et al.*, 1993; Säämanen *et al.*, 2001), lumican (LUM) (Grover *et al.*, 1995; Hassell *et al.*, 1998; Ying *et al.*, 1997), keratocan (Liu *et al.*, 1998; Tasheva *et al.*, 1997, 1998), PRELP (Bengtsson *et al.*, 1995; Grover *et al.*, 1996), and osteoadherin/osteomodulin (Sommarin *et al.*, 1998). Class II SLRPs contain 10 LRRs and the characteristic N-terminal cysteine sequence CX_3CXCX_9C. They are encoded by three exons, with a large central exon encoding almost all 10 LRRs. Except for PRELP, they all carry polylactosamine or keratan sulfate chains in the LRR region and sulfated tyrosine residue in the N-terminal end (see Figure 1 for FM structure).

Class III consists of epiphycan/PG-Lb/DSPG3 (Deere *et al.*, 1996; Johnson *et al.*, 1997), mimecan/osteoglycin (Tasheva *et al.*, 1997, 1999; Funderburgh *et al.*, 1997; Ujita *et al.*, 1995), and opticin/oculoglycan (Friedman *et al.*, 2000; Hobby *et al.*, 2000; Reardon *et al.*, 2000; Takanosu *et al.*, 2001). Class III SLRPs contain only six LRRs and the characteristic N-terminal cysteine sequence CX_2CXCX_6C. They are encoded by seven exons; the last three encode all the LRRs. They all contain sulfated tyrosine residues in the N-terminal end.

The two remaining SLRPs, chondroadherin (Grover *et al.*, 1997; Landgren *et al.*, 1998; Neame *et al.*, 1994) and nyctalopin (Bech-Hansen *et al.*, 2000; Pusch *et al.*, 2000) do not belong to the three previously described classes. Based on their amino acid sequences, they are most closely related to each other than to any other SLRPs (Bech-Hansen *et al.*, 2000). Both contain 11 LRRs and are encoded by 3 exons, but their N-terminal cysteine sequence is different: CX_3CXCX_8C for chondroadherin and CX_3CXCX_6C for nyctalopin.

During evolution, the SLRP family appears to have arisen from several duplication events, which resulted in their current clustered organization on different chromosomes. In humans, for example, DCN (Vetter *et al.*, 1993), LUM (Grover *et al.*, 1995), keratocan (Tasheva *et al.*, 2000a), and epiphycan (Danielson *et al.*, 1999) (Class I, II, II, and III, respectively) map to chromosome 12q23. Asporin (Lorenzo *et al.*, 2001; Henry *et al.*, 2001), osteoadherin, and mimecan (Tasheva *et al.*, 2000b) (Class I, II, and III, respectively) are found on chromosome 9q32. FM (Sztrolovics *et al.*, 1994), PRELP (Grover *et al.*, 1996), and opticin (Hobby *et al.*, 2000) (Class II, II, and III, respectively) locate to chromosome 1q32. BGN (class I) is unique by its location on chromosome X (Xq28) (Fisher *et al.*, 1991). In each cluster, the genes of the class I SLRPs lie 5′ to the genes of class II members with the class II genes lying themselves 5′ to the class III genes (Henry *et al.*, 2001). It is believed that the genes are organized in clusters of four with some genes still to be discovered (Lorenzo *et al.*, 2001).

The expression pattern of the SLRPs varies widely from one proteoglycan to another. In general, class I SLRPs tend to be more ubiquitous than class II members with the distribution of class III SLRPs being the most tissue-specific.

Several SLRPs bind to the collagens type I, II, V, VI, XII, and XIV (Bidanset *et al.*, 1992; Font *et al.*, 1993, 1996; Hedbom and Heinegärd, 1993; Oldberg *et al.*, 1989; Witsch-Prehm *et al.*, 1995; Schönherr *et al.*, 1995a,b; Whinna *et al.*, 1993; Wiberg *et al.*, 2001). By doing so, they modulate collagen fibrillogenesis *in vitro* (see Hocking *et al.*, 1998, for a review). It is generally accepted that (1) horseshoe-shaped SLRPs interact with collagen molecules by their concave surface, and (2) the space inside the horseshoe accommodates a single triple helix of collagen (Weber *et al.*, 1996). Figure 2 shows a 3D structure of DCN including the region believed to bind the collagen. This computerized image was modeled based on the crystal structure of the porcine ribonuclease inhibitor, a molecule that also contains LRRs and shares 18% identical residues with DCN. Structure–function studies showed that the DCN LRR repeats IV and V (Svensson *et al.*, 1995) specifically bind to the C terminus of type I collagen (Keene *et al.*, 2000).

At least three SLRPs (DCN, BGN, and FM) bind to the transforming growth factor beta (TGF-β) (Hildebrand *et al.*, 1994), a multifunctional cytokine involved in inflammation, apoptosis,

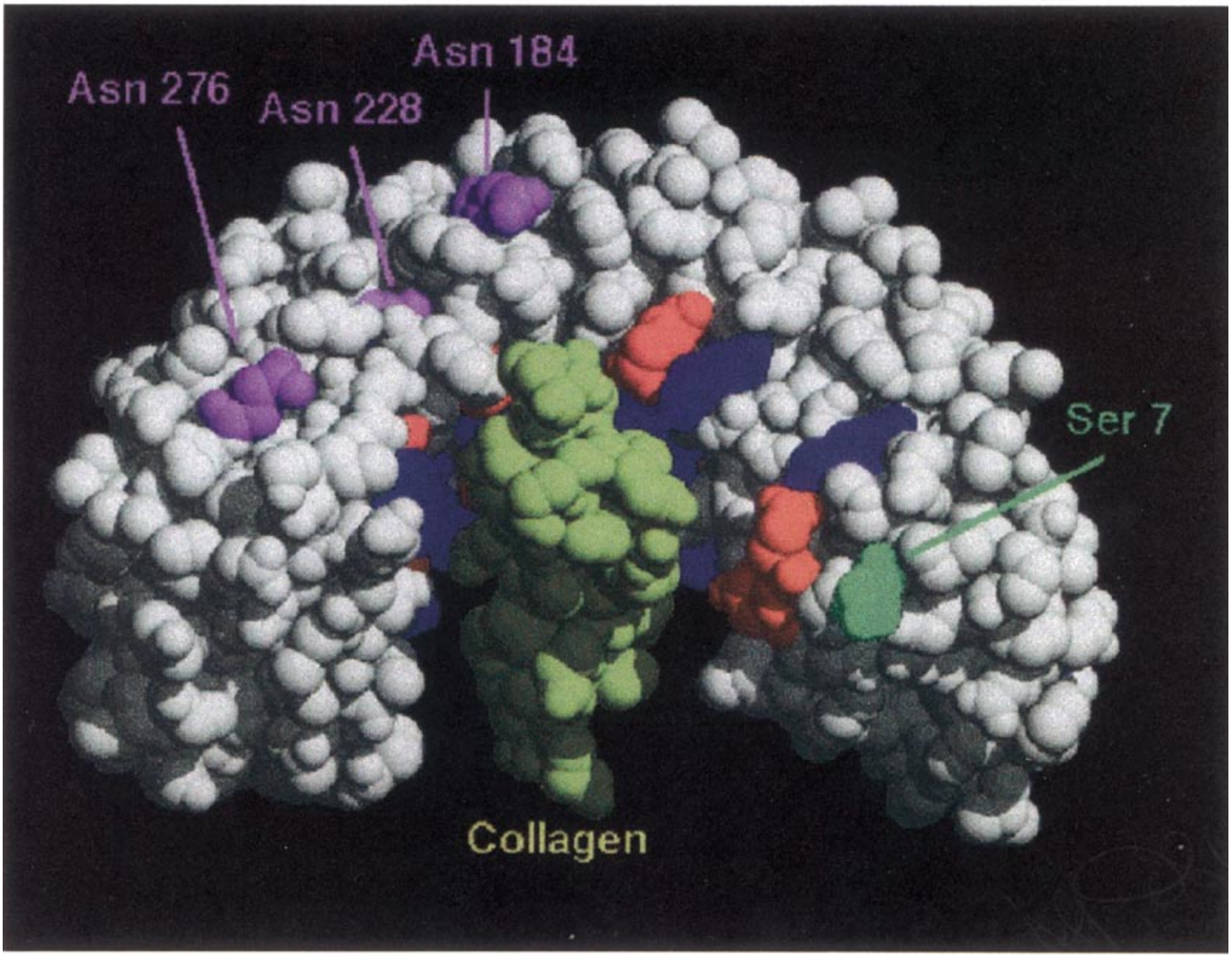

Fig. 2. Modeling of DCN structure based on the crystal structure of the porcine ribonuclease inhibitor. Included with permission of the American Society for Biochemistry and Molecular Biology, Inc., from I.T. Weber, R.W. Harrison, and R.V. Iozzo, 1996, "Model Structure of Decorin and Implications for Collagen Fibrillogenesis," *Journal of Biological Chemistry,* vol. **271**, 31767–31770. DCN (in white) is predicted to bind collagen (shown in green) within the groove of the predicted horseshoe-shaped structure.

cell proliferation, and differentiation (Piek *et al.*, 1999). Due to its TGF-β binding ability, DCN has been successfully used as an antifibrotic agent to overcome the overproduction of TGF-β in experimental glomerulonephritis (Border *et al.*, 1992; Isaka *et al.*, 1996). Recent data suggests that the collagen-bound DCN sequesters TGF-β in the extracellular matrix without inactivating it completely (Hausser *et al.*, 1994; Markmann *et al.*, 2000; Schönherr *et al.*, 1998). Based on *in vitro* studies, a complex picture is beginning to emerge indicating that SLRPs control cell differentiation and proliferation in a cell-specific fashion (Schönherr *et al.*, 2001; Kresse and Schönherr, 2001) with each cell type responding with unique sets of signaling factors (Santra *et al.*, 1997; Iozzo *et al.*, 1999a,b; Xaus *et al.*, 2001).

To better understand the SLRP functions *in vivo*, four SLRPs (BGN, DCN, FM, and LUM) have been disrupted by gene targeting. In addition, double SLRP-deficient mice (deficient in BGN and DCN, FM and LUM, or BGN and FM) have also been generated by interbreeding the single SLRP-deficient mice. The rest of this review focuses on what we have learned about SLRP biology from these animal models.

SLRP single-deficient mice

BGN-deficient mouse

BGN is highly expressed in bone (Bianco *et al.*, 1990). For several years, it was suspected to be involved in bone growth because patients with Turner syndrome (i.e., females that lack a second X chromosome) have short stature and low levels of BGN, whereas patients with supernumerary X chromosomes present increased limb length and levels of BGN (Vetter *et al.*, 1993). The targeted disruption of BGN confirmed that it was involved in the regulation of postnatal skeletal growth (Xu *et al.*, 1998). BGN-deficient bones grow more slowly than their wild-type counterparts, and by 9 months BGN-deficient femurs are significantly shorter than wild-type femurs. BGN knockout mice also fail to achieve peak bone mass due to a

decrease in bone formation explained by a lower osteoblast number and a lower osteoblast activity (Xu *et al.*, 1998). The phenotypical analysis of BGN-deficient mice demonstrates that BGN is a positive regulator of bone formation that controls peak bone mass. Peak bone mass, achieved by skeletal growth, is the most critical risk factor for human osteoporosis. By its inability to achieve a high peak bone mass, the BGN knockout mouse thus constitutes a good animal model for studying osteoporosis.

The cellular and molecular mechanisms leading to osteoporosis in the absence of BGN have been partially identified (Chen *et al.*, 2002). *In vitro* experiments demonstrate that with age, the number of bone marrow stroma cells (BMSCs; the osteogenic precursors) decreases more rapidly in the BGN knockout than in the wild type (Chen *et al.*, 2002). Though the number of BMSCs isolated from 6-week-old BGN knockout mice is not statistically different from wild-type controls, the difference becomes statistically significant at 3 months and grows even larger by 6 months.

Other experiments indicate that the BGN deficiency also impairs the metabolic activity of the BMSCs. BGN-deficient BMSCs are less responsive to exogenous TGF-β and show an increased rate of apoptosis, two possible explanations for the lower number of BMSCs observed at 3 and 6 months in the BGN knockout. BGN-deficient BMSCs also produce less type I collagen, indicating that the composition of the extracellular matrix may be regulated by specific matrix components like BGN. Any one of the defects in BMSCs (lower number, decrease response to growth factors, increased rate of apoptosis, synthesis of an abnormal extracellular matrix) or any combination could contribute to and/or explain the osteoporosis developed by the BGN knockout mice *in vivo*.

Reminiscent of the altered bone phenotype, 1 day after birth, a broader than normal metadentin is observed in the BGN-deficient first molar, along with an altered and heterogeneous dentin mineralization (Goldberg *et al.*, 2002). Structural defects are also observed in the forming enamel. In addition, the thickness of the forming enamel is dramatically increased. These results suggests that BGN exerts a negative control on enamel formation and that its absence derepressed ameloblast synthetic and secretory pathways. Altogether, these results stressed the important role played by BGN in all mammalian mineralized tissues.

In addition, the BGN-deficient mice also develop osteoarthritis and ectopic tendon ossification (Ameye *et al.*, 2002a) Both may result from joint instability and mechanically compromised tendons (Ameye *et al.*, 2002a,b). The BGN deficiency induces morphological changes in collagen fibrils from a wide variety of tissues, including tendons (Goldberg *et al.*, 2002; Ameye *et al.*, 2002a; Corsi *et al.*, 2002) (Table II). In the absence of BGN, irregular fibrils with a cross-sectional contour presenting notches and protuberances are frequently observed in type I collagen-rich matrices, such as skin, bone, and tendons. Longitudinal sections of collagen fibrils in BGN knockout also demonstrate marked diameter variability along individual fibril axes.

In addition to these qualitative morphological defects, the absence of BGN introduces quantitative variations in the range, mean and distribution profiles of the collagen fibril diameters compared to the wild type (Goldberg *et al.*, 2002; Ameye *et al.*, 2002a; Corsi *et al.*, 2002). Interestingly, although the morphological defects are the same in all extracellular matrices, the quantitative alterations are tissue-specific (Corsi *et al.*, 2002). For example, the BGN deficiency increases the average fibril diameter in bone and skin dermis, while it decreases it in the patella and tail tendons (Ameye *et al.*, 2002a; Corsi *et al.*, 2002). The tissue-specific effects of the BGN deficiency could result from tissue differences in the amount and/or temporal expression of BGN during collagen fibrillogenesis or from a modulation of the control exerted by BGN on collagen fibrillogenesis by other local components of the extracellular matrix. Though *in vitro* the collagen binding ability of BGN and the inferred effect of BGN on collagen fibrillogenesis is controversial (Hocking *et al.*, 1998), the *in vivo* data clearly establish that BGN plays a critical role in collagen fibrillogenesis.

BGN also binds to α-dystroglycan, a component of the dystrophin-associated protein complex localized in the muscle cell membrane (Bowe *et al.*, 2000). BGN binding appears to occur through the glycosaminoglycan chains, leaving open the possibility that other proteoglycans may also interact with dystroglycan. Mice deficient in BGN display a mild muscular dystrophic phenotype characterized by membrane disruption and cell death of a subpopulation of myofibers (Rafii *et al.*, 2000). These results suggest that BGN may play a role in muscular dystrophies and in the future treatments of these pathologies.

DCN-deficient mouse

The targeted disruption of DCN was the first *in vivo* demonstration of the importance of the SLRPs for proper collagen fibrillogenesis (Danielson *et al.*, 1997). In the absence of DCN, the collagen fibrils of the skin and tail tendon show the same qualitative morphological defects as in the absence of BGN (Corsi *et al.*, 2002; Danielson *et al.*, 1997). The DCN deficiency also introduces tissue-specific variations in the range, mean, and distribution profiles of the collagen fibril diameters compared to the wild type. In some tissues, these variations are distinct from those observed in the absence of BGN (Corsi *et al.*, 2002) (Table II). Consistent with the high level of expression of DCN in the dermis, DCN-deficient mice have fragile skin, characterized by a markedly reduced tensile strength and a thinner than normal dermis. The collagen alterations observed in the skin of the DCN knockouts are probably responsible for the reduced mechanical properties of the skin because skin strength correlates directly with the characteristics of the collagen fibril network (Dombi *et al.*, 1993). The phenotype of the DCN- deficient mice, including the collagen defects, closely mimic the cutaneous defects observed in the human Ehlers-Danlos syndrome (EDS) (Danielson *et al.*, 1997), a clinically and genetically heterogeneous connective tissue disorder characterized by skin hyperextensibility, joint hypermobility, and tissue fragility (Mao and Bristow, 2001).

Both DCN and BGN single deficiencies introduce changes in collagen fibril size and shape in bone (Table II) (Corsi *et al.*, 2002). However, in contrast to BGN deficiency, DCN deficiency does not affect bone mass and does not appear to lead to any other major phenotypic changes in bone at the histological or macroscopic level (Corsi *et al.*, 2002). Despite their high level of homology (57% identity at the amino acid level), BGN and DCN have distinct functions *in vivo*. These functional specificities may partially result from differences in the glycosaminoglycan chains: BGN most often has two chondroitin or dermatan sulfate chains, whereas DCN contains only one.

In addition to modulating the morphology of the collagen fibrils, DCN deficiency also induces changes in collagen

Table II. Comparisons of the mean diameters and diameter ranges of collagen type I fibrils between wild-type and SLRP-deficient tissues

Genotypes	Tissue	Diameters (nm)	Ranges	*N*	Age	Reference
BGN-deficient / wild type	Skin	104 / 62	21–316 / 20–148	2000	2 months	Corsi *et al.*, 2002
	Femur	33 / 29	10–84 / 10–67	2000	2 months	Corsi *et al.*, 2002
	Tail tendon	107 / 118	13–435 / 13–322	2000	2 months	Corsi *et al.*, 2002
	Proximal predentin	16 / 8	NA	50	1 day	Goldberg *et al.*, 2002
	Central predentin	34 / 47	NA	50	1 day	Goldberg *et al.*, 2002
	Distal predentin	36 / 52	NA	50	1 day	Goldberg *et al.*, 2002
	Quadriceps tendon	105 / 150	35–199 / 30–261	250	3 months	Ameye *et al*., 2002a
DCN-deficient / wild type	Skin	87 / 62	17–224 / 20–148	2000	2 months	Corsi *et al.*, 2002
		119 / 116	40–260 / 40–180	780		Danielson *et al*., 1999
	Femur	26 / 29	10–84 / 10–60	2000	2 months	Corsi *et al.*, 2002
	Tail tendon	128 / 150	17–452 / 13–322	2000	2 months	Corsi *et al.*, 2002
FM-deficient / wild type	Achilles tendon	128 / 152	NA	1600		Svensson *et al.*, 1999
	Flexor tendon	71 / 64	30–120 / 30–100	>2000	4 days	Ezura *et al.*, 2000
		84 / 102	30–170 / 30–240	>2000	10 days	Ezura *et al.*, 2000
		178 / 195	30–410 / 30–370	>2000	1 months	Ezura *et al.*, 2000
		175 / 223	30–410 / 30–410	>2000	3 months	Ezura *et al.*, 2000
	Quadriceps tendon	95 / 150	32–245 / 30–261	250	3 months	Ameye *et al*., 2000a
LUM-deficient / wild type	Tail dermis	107 / 90	33–250 / 50–150	150		Chakravarti *et al.*, 1998
	Cornea	47 / 30	20–235 / 10–42	250		Chakravarti *et al.*, 1998
	Anterior stroma of the cornea	31 / 32	17–48 / 14–46	NA	7.5 months	Charkravarti *et al*., 2000
	Posterior stroma of the cornea	79 / 41	21–100 / 22–63	NA	7.5 months	Charkravarti *et al*, 2000
	Flexor tendon	72 / 64	30–110 / 30–100	>2000	4 days	Ezura *et al.*, 2000
		98 / 102	30–210 / 30–240	>2000	10 days	Ezura *et al.*, 2000
		189 / 195	30–330 / 30–370	>2000	1 month	Ezura *et al.*, 2000
		228 / 223	30–410 / 30–410	>2000	4 months	Ezura *et al.*, 2000
BGN/DCN double / wild type	Skin	92 / 62	23–423 / 20–148	2000	2 months	Corsi *et al.*, 2002
	Femur	34 / 29	13–92 / 10–67	2000	2 months	Corsi *et al*., 2002
	Tail tendon	102 / 118	14–439 / 13–322	2000	2 months	Corsi *et al*., 2000
FM/LUM double / wild type	Flexor tendon	87 / 64	30–160 / 30–100	>2000	4 days	Ezura *et al.*, 2000
		104 / 102	30–210 / 30–240	>2000	10 days	Ezura *et al.*, 2000
		166 / 195	30–330 / 30–370	>2000	1 months	Ezura *et al.*, 2000
		142 / 223	30–360 / 30–410	>2000	4 months	Ezura *et al.*, 2000
BGN/FM double / wild type	Quadriceps tendon	90 / 150	24–223 / 30–261	250	3 months	Ameye *et al*., 2002a

Alterations in mean diameter and diameter range of collagen type I fibrils in absence of biglycan (BGN), decorin (DCN), fibromodulin (FM), and lumican (LUM). Comparisons were made between the various knockout mice to their wild-type littermates (BGN-deficient/wild type, for example). The first column lists the genotypes of the mice used for the ultrastructural analysis. Tissue source used for the analysis is listed in the second column, and the diameter for the two genotypes being compared is shown in the third column. The range in diameter size for a specific genotype and tissue is shown in the fourth column and the number of samples tested (*N*) shown in the fifth column. The age of the mice that the tissue was taken from is shown in the sixth column. The original citation used to assemble the data is found in the last column. NA= not available.

orientation. In the absence of DCN, the collagen fibrils in the periodontal ligament display a random orientation instead of their usual parallel orientation (Hakkinen *et al.*, 2000).

Besides its action on collagen fibrillogenesis, DCN is also known to control cell division (Santra *et al.*, 1997). *In vitro*, the ectopic expression of DCN in a wide variety of malignant cell lines and in normal fibroblasts stops cell growth (Hakkinen *et al.*, 2000; Santra *et al.*, 1997). The inhibitory action of DCN on cell division has been confirmed *in vivo* by the data gathered from DCN-deficient mice. Compared to wild-type mice, the number of fibroblasts in the periodontal ligament is doubled in DCN-deficient mice (Hakkinen *et al.*, 2000). This hyper-cellularity probably results from an increased proliferation in the absence of inhibitory signals from DCN. In agreement with

this interpretation, the lack of DCN also accelerates lymphoma tumorigenesis in a mouse model predisposed to cancer (Iozzo *et al.*, 1999b). In parallel to its regulatory action on cell division, DCN may also limit apoptosis. Indeed, DCN-deficient mice subject to unilateral ureteral obstruction have increased apoptosis (and collagen turnover) indicating that DCN may have a protective function in acquired tubulointerstitial fibrosis (Schaefer *et al.*, 2002).

The DCN-deficient mice were also instrumental to demonstrate the importance of DCN in Lyme disease. Lyme disease is an infectious disease caused by the tick-borne spirochete *Borrelia burgdorferi* (Steere, 2001). Spirochetes deposited by the ticks in the dermis bind to DCN (Guo *et al.*, 1995). The DCN-deficient mice display an increased resistance to Lyme disease, suggesting that DCN could be a limiting factor as a substrate for the adherence of *B. burgdorferi* in the dermis (Brown *et al.*, 2001).

FM-deficient mouse

FM is relatively abundant in tendons. Mice deficient in FM are predisposed to ectopic tendon ossification and develop osteoarthritis (Ameye *et al.*, 2002a). At the histological level, FM-deficient mice have abnormal and less numerous collagen fibril bundles in the tail (Svensson *et al.*, 1999). At the ultrastructural level, collagen fibrils with irregular cross sections are observed (Ameye *et al.*, 2002a; Svensson *et al.*, 1999). Compared to wild type, the average fibril diameter is decreased both in Achilles and patella tendons due to a higher number of small fibrils in the fibril population (Ameye *et al.*, 2002a; Svensson *et al.*, 1999). The ratio of LUM to FM was estimated to be 1:3 in wild-type tendons. In absence of FM, the amount of LUM protein in tail tendon is multiplied by a factor of four despite a decrease at the mRNA level (Svensson *et al.*, 1999). Therefore, it was hypothesized that in absence of FM, LUM binds to the sites normally used by FM, suggesting that LUM and FM compete for the same binding sites on collagen fibrils. This was later confirmed by binding competition experiments (Svensson *et al.*, 2000).

LUM-deficient mouse

LUM is a major component of the cornea. Mice deficient in LUM develop progressive corneal opacification with age, demonstrating that although LUM is not necessary for embryonic corneal development, it is essential for postnatal corneal maturation (Chakravarti *et al.*, 1998). Absence of LUM results in irregular, thicker than normal, loosely packed collagen fibrils associated with a dramatic disruption in the lamellar organization of the fibrils (Table II) (Chakravarti *et al.*, 1998, 2000; Quantock *et al.*, 2001). These collagen defects are mainly restricted to the posterior part of the cornea, where the mature collagen fibrils are located and where LUM is more highly expressed in normal animals (Chakravarti *et al.*, 2000). The structural alteration of the collagen fibrils is probably directly responsible for the corneal opacification, as corneal transparency arises from minimal scattering of light by a lattice arrangement of uniformly thin, regularly interspaced collagen fibrils (Maurice, 1957). Supporting this hypothesis, *in vivo* confocal microscopy demonstrates that the posterior stroma of the cornea, where the collagen defects are mainly concentrated, is highly reflective to light (Chakravarti *et al.*, 2000; Jester *et al.*, 2001).

In addition, LUM-deficient mice also develop EDS-like skin laxity. Compared to wild type, the LUM-deficient mice display a 86% reduction in skin tensile strength, an increased incidence of skin lesions, along with a disorganized abnormally loose dermis containing altered collagen fibrils (Table II) (Chakravarti *et al.*, 1998).

Double jeopardy: mice deficient in more than one SLRP

The structural similarities and partially overlapping tissue distributions of the SLRPs suggested that different SLRPs might share redundant functions and that partial rescue and/or compensation mechanisms by other members of the family might take place in singly SLRP-deficient mice. In a rescue situation, the total amount of the extracellular matrix components would stay the same whereas in a compensation situation, the amount of one or several molecules would be increased. An increased protein level could be achieved either by an increased level of expression (regulated at the transcriptional or translational level) or by a decreased rate of degradation. The increased amount of LUM in FM-deficient tendon was the first evidence of the existence of compensation mechanisms between SLRPs (Svensson *et al.*, 1999). In this context and in order to further investigate the *in vivo* functional relationships between SLRPs, double SLRP-deficient mice were generated. Double deficient mice are critical tools to convincingly demonstrate the existence of redundant functions between molecules because if such redundant functions exist, the effects of a double deficiency will be synergetic instead of additive.

BGN/DCN double knockout

BGN and DCN were obvious candidates to generate a double SLRP-deficient mouse because of the high level of similarity between these two Class I SLRPs. The phenotype of the double knockout is severe. They cannot breed, and the number of double-deficient animals obtained for heterozygous breeders is much lower than the expected Mendelian frequencies (Corsi *et al.*, 2002).

The phenotypic analysis of the double knockout reveals that the bone phenotype is more severe and develops earlier than in the BGN single-deficient mice (Corsi *et al.*, 2002). At 2 months, when a decrease in bone mass is barely detectable in the BGN-deficient animal, cortical and trabecular bone mass are severely reduced in the double knockout compared to the wild type. Thus the effects of the BGN and DCN double deficiency are synergistic in bone even if bone is not affected by the DCN deficiency. Despite their distinct functions (demonstrated by the distinct phenotypes developed by the BGN and DCN single-deficient mice), BGN and DCN are therefore still sufficiently similar to partly rescue or compensate their absence in the singly deficient mice. In addition to the bone phenotype, the double knockout displays skin fragility reminiscent of the skin phenotype observed in the DCN-deficient mice.

Not surprisingly, the ultrastructure of the collagen fibrils in absence of BGN and DCN is altered (Table II) (Corsi *et al.*, 2002). The most dramatic changes are observed in bone. In single-deficient animals, the collagen fibrils conserve a roughly circular cross-section despite the presence of protuberances

and notches. In double-deficient animals, the circular aspect of the cross-sections is completely lost and replaced by a hieroglyphic-like morphology due to highly interconnected fibrils displaying a wide variety of shapes.

Altogether, the BGN/DCN double-knockout phenotype is reminiscent of a specific subtype of human EDSs, the progeroid variant (OMIM 130070) (Corsi *et al.*, 2002). Fibroblasts from this variant have a defective xylosylprotein 4-β-galactosyltransferase I, an enzyme necessary for the synthesis of glycosaminoglycan chains (Quentin *et al.*, 1990). The fact that the absence of BGN and DCN in the mouse mimics a human syndrome in which glycosaminoglycan-free DCN and BGN are produced underlies the potential functional importance of the glycosaminoglycan chains in BGN and DCN.

FM/LUM double knockout

FM and LUM double-deficient mice were generated to define more precisely the role of these two Class II SLRPs in the regulation of collagen fibrillogenesis in (flexor) tendon development (Ezura *et al.*, 2000). Both SLRPs compete for the same binding sites on the collagen fibrils with FM having the higher affinity (Svensson *et al.*, 2000). The collagen defects observed in the FM and LUM single- and double-deficient mice were compared to each other at different key developmental steps (initial fibril assembly, fibril growth initiation, maturation), while in parallel the temporal expression of FM and LUM in wild-type tendons was determined (Pellegata *et al.*, 2000).

Collagen defects similar to those reported herein are observed during the flexor tendon development of these three SLRP knockouts. In the absence of LUM, the collagen defects are observed early but tend to disappear with time. In the absence of FM, defects similar to those observed in the absence of LUM are observed early but become more severe with time. In the double-deficient mice, the phenotype is more severe than the single-deficient phenotypes early but comparable with the FM single-deficient mice at maturation. It appears that LUM and FM both influence the initial assembly of the fibril, whereas FM, in addition, influences the fibril growth and its maturation. During the wild-type flexor tendon development, the amounts of LUM and FM peak early before decreasing significantly, with LUM being down-regulated before FM. These temporal expression profiles of LUM and FM fit the temporal evolution of the collagen defects observed in the FM and LUM single- and double-deficient mice. These results demonstrate that FM and LUM act at different developmental/maturation stages and have distinct functions in the regulation of collagen fibrillogenesis.

BGN/FM double knockout

BGN and FM are coexpressed in tendons, cartilage, and bone. BGN/FM double knockouts were generated to study potential *in vivo* interactions between Class I and II SLRPs. The double-deficient mice develop sequentially and progressively gait impairment, ectopic tendon ossification, and severe premature osteoarthritis (Ameye *et al.*, 2002a). In several instances, compared to the effects of the single deficiencies, the effects of the double deficiency are synergistic, indicating the existence of rescuing/compensatory mechanisms in single mutants and the presence of redundant functions between BGN and FM (Ameye *et al.*, 2002a).

The phenotype probably results primarily from collagen defects in tendons. In addition to displaying abnormal collagen fibrils, the BGN/FM-deficient tendons have a higher plasticity than their wild-type counterparts, judged by biomechanical testing (Ameye *et al.*, 2002a). The reduced stiffness in tendon is thought to destabilize the knee joint and allow abnormal impact during joint movement, leading eventually to cartilage erosion. In parallel, joint instability is thought (but not proven) to create abnormal mechanical tensions within tendons that trigger their ossification. Supporting this hypothesis, increased use of the joints, induced by treadmill running, amplifies both tendon ossification and osteoarthritis in the double-deficient mice (Ameye *et al.*, 2002a). Considering the fact that TGF-β is implicated both in ossification and cartilage metabolism, and that it binds to BGN and FM, it is additionally tempting to speculate that abnormalities in TGF-β distribution and function in the BGN/FM knockout contribute to the ectopic ossification and cartilage destruction. The BGN/FM double knockout constitutes a valuable animal model for spontaneous osteoarthritis, characterized by an early onset and a rapid progression of the disease.

Conclusion and future directions

The major advance brought by the generation of SLRP-deficient mice in our understanding of SLRP function *in vivo* is the crucial role played by SLRPs in the control of collagen fibrillogenesis. All four tested SLRP deficiencies lead to defects in collagen type I fibrils and most of the diseases developed by the deficient mice appear to result from these collagen defects. Although the collagen phenotypes developed by the different single-deficient SLRP mice are distinct, implying distinct *in vivo* function for each SLRP, the existence of rescuing/compensation mechanisms in the double-deficient mice indicates some functional overlap within the SLRP family. Taken together, the diverse collagen phenotypes demonstrate a cooperative, sequential, timely orchestrated action of the SLRPs that altogether shape the architecture and mechanical properties of the collagen matrix.

SLRP-deficient mice develop a wide array of diseases that include osteoporosis, osteoarthritis, muscular dystrophy, EDS, and corneal diseases. Not unexpectedly, the disease(s) developed by the different SLRP-deficient mice reflects the major physiological site(s) of expression of the targeted SLRP(s). The diseases developed by these new animal models indicates that mutations in SLRPs may be part of yet undiagnosed predisposing genetic factors for these diseases.

The SLRP family is rapidly growing, constantly creating new possibilities for future investigations. Recently, mutations in two SLRPs have been shown to be responsible for two human ocular disorders. Mutations in nyctalopin result in congenital stationary night blindness, a retinal disorder characterized by abnormal night vision (Bech-Hansen *et al.*, 2000; Pusch *et al.*, 2000), whereas mutations in keratocan result in cornea plana, a corneal disorder characterized by a flattened forward convex curvature of the cornea and a decreased refraction (Pellegata *et al.*, 2000). Generation of nyctalopin and keratocan "knock-in" mouse models harboring one of these mutations will constitute valuable tools to unravel the molecular pathways underlying these diseases. Other obvious future research

directions include the generation of knock-in mice harboring mutations leading to the synthesis of nonproteoglycan forms of SLRP to investigate the functional role played by the glycosaminoglycan chains or the generation of SLRP/TGF-β double genetically modified mice to study the *in vivo* inter-relationships between SLRPs and TGF-βs. Finally, the phenotypic characterization of the available deficient mice is only partially completed. Further analysis will probably bring new exciting insights into their *in vivo* functions.

Abbreviations

BGN, biglycan; BMSCs, bone marrow stroma cells; DCN, decorin; EDS, Ehlers-Danlos syndrome; FM, fibromodulin; LRR, leucine rich repeat; LUM, lumican; SLRP, small leucine rich proteoglycan; TGF, transforming growth factor.

References

Ameye, L., Aria, D., Jepsen, K., Oldberg, A., Xu, T., and Young, M. (2002a) Abnormal collagen fibrils in tendons of biglycan/fibromodulin deficient mice lead to gait impairment, ectopic ossification and osteoarthritis. *FASEB J.*, **16**, 673–680.

Ameye, L., Young, M.F., and Chen, X.D. (2002b) Decorin. In: Creighton, T. (ed.), *Wiley encyclopedia of molecular medecine*. Wiley, New York, pp. 1008–1010.

Antonsson, P., Heinegärd, D., and Oldberg, Å. (1993) Structure and deduced amino acid sequence of the human fibromodulin gene. *Biochim. Biophys. Acta*, **1174**, 204–206.

Bech-Hansen, N.T., Naylor, M.J., Maybaum, T.A., Sparkes, R.L., Koop, B., Birch, D.G., Bergen, A.A., Prinsen, C.F., Polomeno, R.C., Gal, A., and others. (2000) Mutations in NYX, encoding the leucine-rich proteoglycan nyctalopin, cause X-linked complete congenital stationary night blindness. *Nature Genet.*, **26**, 319–323.

Bengtsson, E., Neame, P., Heinegärd, D., and Sommarin, Y. (1995) The primary structure of a basic leucine-rich repeat protein, PRELP, found in connective tissues. *J. Biol. Chem.*, **270**, 25639–25644.

Bianco, P., Fisher, L., Young, M., Termine, J., and Gehron Robey, P. (1990) Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues. *J. Histochem. Cytochem.*, **38**, 1549–1563.

Bidanset, D.J., Guidry, C., Rosenberg, L.C., Choi, H.U., Timpl, R., and Höök, M. (1992) Binding of the proteoglycan decorin to collagen type VI. *J. Biol. Chem.*, **267**, 5250–5256.

Border, W.A., Noble, N.A., Yamamoto, T., Harper, J.R., Yamaguchi, Y., Pierschbacher, M.D., and Ruoslahti, E. (1992) Natural inhibitor of transforming growth factor-beta protects against scarring in experimental kidney disease. *Nature*, **360**, 361–364.

Bowe, M.A., Mendis, D.B., and Fallon, J.R. (2000) The small leucine-rich repeat proteoglycan biglycan binds to alpha-dystroglycan and is upregulated in dystrophic muscle. *J. Cell Biol.*, **148**, 801–810.

Brown, E.L., Wooten, R.M., Johnson, B.J., Iozzo, R.V., Smith, A., Dolan, M.C., Guo, B.P., Weis, J.J., and Höök, M. (2001) Resistance to Lyme disease in decorin-deficient mice. *J. Clin. Invest.*, **107**, 845–852.

Chakravarti, S., Magnuson, T., Lass, J., Jepsen, K., LaMantia, C., and Carroll, H. (1998) Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican. *J. Cell Biol.*, **141**, 1277–1286.

Chakravarti, S., Petroll, W.M., Hassell, J.R., Jester, J.V., Lass, J.H., Paul, J., and Birk, D.E. (2000) Corneal opacity in lumican-null mice: defects in collagen fibril structure and packing in the posterior stroma. *Invest. Ophthalmol. Vis. Sci.*, **41**, 3365–3373.

Chen, X.-D., Shi, S., Xu, T., Gehron-Robey, P., and Young, M. (2002) Age-related osteoporosis in biglycan deficient mice is related to defects in bone marrow stromal cells. *J. Bone Min. Res.* (in press).

Corsi, A., Xu, T., Chen, X.-D., Boyde, A., Liang, J., Mankani, M., Sommer, B., Iozzo, R.V., Eichstetter, I., Gehron Robey, P., and others. (2002) Phenotypic effects of biglcyan deficiency are linked to collagen fibril abnormalities, are synergized by decorin deficiency, an mimic Ehlers-Danlos-like changes in bone and other connective tissues. *J. Bone Min. Res.* (in press).

Danielson, K., Baribault, H., Holmes, D., Graham, H., Kadler, K., and Iozzo, R. (1997) Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J. Cell Biol.*, **136**, 729–749.

Danielson, K., Fazzio, A., Cohen, I., Cannizzaro, L., Eichstetter, I., and Iozzo, R. (1993) The human decorin gene: intron-exon organization, discovery of two alternatively spliced exons in the 5′ untranslated region, and mapping of the gene to chromosome 12q23. *Genomics*, **15**, 146–160.

Danielson, K.G., Siracusa, L.D., Donovan, P.J., and Iozzo, R.V. (1999) Decorin, epiphycan, and lumican genes are closely linked on murine chromosome 10 and are deleted in lethal steel mutants. *Mamm. Gen.*, **10**, 201–203.

Deere, M., Johnson, J., Garza, S., Harrison, W.R., Yoon, S.J., Elder, F.F., Kucherlapati, R., Höök, M., and Hecht, J.T. (1996) Characterization of human DSPG3, a small dermatan sulfate proteoglycan. *Genomics*, **38**, 399–404.

Dombi, G.W., Haut, R.C., and Sullivan, W.G. (1993) Correlation of high-speed tensile strength with collagen content in control and lathyritic rat skin. *J. Surg. Res.*, **54**, 21–28.

Ezura, Y., Chakravarti, S., Oldberg, A., Chervoneva, I., and Birk, D.E. (2000) Differential expression of lumican and fibromodulin regulate collagen fibrillogenesis in developing mouse tendons. *J. Cell Biol.*, **151**, 779–787.

Fisher, L.W., Heegaard, A.M., Vetter, U., Vogel, W., Just, W., Termine, J.D., and Young, M.F. (1991) Human biglycan gene. Putative promoter, intron-exon junctions, and chromosomal localization. *J. Biol. Chem.*, **266**, 14371–14377.

Fisher, L.W., Termine, J.D., and Young, M.F. (1989) Deduced protein sequence of bone small proteoglycan I (biglycan) shows homology with proteoglycan II (decorin) and several nonconnective tissue proteins in a variety of species. *J. Biol. Chem.*, **264**, 4571–4576.

Font, B., Aubert-Foucher, E., Goldschmidt, D., Eichenberger, D., and van der Rest, M. (1993) Binding of collagen XIV with the dermatan sulfate side chain of decorin. *J. Biol. Chem.*, **268**, 25015–25018.

Font, B., Eichenberger, D., Rosenberg, L.M., and van der Rest, M. (1996) Characterization of the interactions of type XII collagen with two small proteoglycans from fetal bovine tendon, decorin and fibromodulin. *Matrix Biol.*, **15**, 341–348.

Friedman, J.S., Ducharme, R., Raymond, V., and Walter, M.A. (2000) Isolation of a novel iris-specific and leucine-rich repeat protein (oculoglycan) using differential selection. *Invest. Ophthalmol. Vis. Sci.*, **41**, 2059–2066.

Funderburgh, J.L., Corpuz, L.M., Roth, M.R., Funderburgh, M.L., Tasheva, E.S., and Conrad, G.W. (1997) Mimecan, the 25-kDa corneal keratan sulfate proteoglycan, is a product of the gene producing osteoglycin. *J. Biol. Chem.*, **272**, 28089–28095.

Goldberg, M., Rapoport, O., Septier, D., Palmier, K., R, H., embery, G., Young, M., and Ameye, L. (2002) Proteoglycans in predentin: the last 15 micrometers before mineralization. *Conn. Tiss. Res.* (in press).

Grover, J., Chen, X., Korenberg, J., and Roughley, P. (1995) The human lumican gene: organization, chromosomal location and expression in articular cartilage. *J. Biol. Chem.*, **270**, 21942–21949.

Grover, J., Chen, X.N., Korenberg, J.R., Recklies, A.D., and Roughley, P.J. (1996) The gene organization, chromosome location, and expression of a 55-kDa matrix protein (PRELP) of human articular cartilage. *Genomics*, **38**, 109–117.

Grover, J., Chen, X.N., Korenberg, J.R., and Roughley, P.J. (1997) The structure and chromosome location of the human chondroadherin gene (CHAD). *Genomics*, **45**, 379–385.

Guo, B.P., Norris, S.J., Rosenberg, L.C., and Höök, M. (1995) Adherence of *Borrelia burgdorferi* to the proteoglycan decorin. *Infect. Immun.*, **63**, 3467–3472.

Hakkinen, L., Strassburger, S., Kahari, V.M., Scott, P.G., Eichstetter, I., Lozzo, R.V., and Larjava, H. (2000) A role for decorin in the structural organization of periodontal ligament. *Lab. Invest.*, **80**, 1869–1880.

Hassell, J.R., Rada, J., Cornuet, P., Vergnes, J.P., and Kinchington, P.R. (1998) Gene structure of chick lumican and identification of the first exon. *Biochim. Biophys. Acta*, **1397**, 119–125.

Hausser, H., Groning, A., Hasilik, A., Schönherr, E., and Kresse, H. (1994) Selective inactivity of TGF-beta/decorin complexes. *FEBS Lett.*, **353**, 243–245.

Hedbom, E. and Heinegärd, D. (1993) Binding of fibromodulin and decorin to separate sites on fibrillar collagens. *J. Biol. Chem.*, **268**, 27307–273112.

Henry, S.P., Takanosu, M., Boyd, T.C., Mayne, P.M., Eberspaecher, H., Zhou, W., de Crombrugghe, B., Höök, M., and Mayne, R. (2001) Expression pattern and gene characterization of asporin, a newly discovered member of the leucine-rich repeat protein family. *J. Biol. Chem.*, **276**, 12212–12221.

Hildebrand, A., Romaris, M., Rasmussen, L., Heinegärd, D., Twardzik, D., Borders, W., and Ruoslathi, E. (1994) Interaction of the small interstitial proteoglycans, decorin and fibromodulin with transforming growth factor b. *Biochem. J.*, **302**, 527–534.

Hobby, P., Wyatt, M.K., Gan, W., Bernstein, S., Tomarev, S., Slingsby, C., and Wistow, G. (2000) Cloning, modeling, and chromosomal localization for a small leucine- rich repeat proteoglycan (SLRP) family member expressed in human eye. *Mol. Vis.*, **6**, 72–78.

Hocking, A.M., Shinomura, T., and McQuillan, D.J. (1998) Leucine-rich repeat glycoproteins of the extracellular matrix. *Matrix Biol.*, **17**, 1–19.

Iozzo, R. (1999) The biology of the small leucine-rich proteoglycans. *J. Biol. Chem.*, **274**, 18843–18846.

Iozzo, R.V. (1998) Matrix proteoglycans: from molecular design to cellular function. *Annu. Rev. Biochem.*, **67**, 609–652.

Iozzo, R. and Murdoch, A. (1996) Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. *FASEB J.*, **10**, 598–614.

Iozzo, R.V., Moscatello, D.K., McQuillan, D.J., and Eichstetter, I. (1999a) Decorin is a biological ligand for the epidermal growth factor receptor. *J. Biol. Chem.*, **274**, 4489–4492.

Iozzo, R.V., Chakrani, F., Perrotti, D., McQuillan, D.J., Skorski, T., Calabretta, B., and Eichstetter, I. (1999b) Cooperative action of germ-line mutations in decorin and p53 accelerates lymphoma tumorigenesis. *Proc. Natl Acad. Sci. USA*, **96**, 3092–3097.

Isaka, Y., Brees, D.K., Ikegaya, K., Kaneda, Y., Imai, E., Noble, N.A., and Border, W.A. (1996) Gene therapy by skeletal muscle expression of decorin prevents fibrotic disease in rat kidney. *Nat. Med.*, **2**, 418–423.

Jester, J.V., Ghee Lee, Y., Li, J., Chakravarti, S., Paul, J., Petroll, W.M., and Dwight Cavanagh, H. (2001) Measurement of corneal sublayer thickness and transparency in transgenic mice with altered corneal clarity using *in vivo* confocal microscopy. *Vis. Res.*, **41**, 1283–1290.

Johnson, H.J., Rosenberg, L., Choi, H.U., Garza, S., Höök, M., and Neame, P.J. (1997) Characterization of epiphycan, a small proteoglycan with a leucine-rich repeat core protein. *J. Biol. Chem.*, **272**, 18709–18717.

Keene, D.R., San Antonio, J.D., Mayne, R., McQuillan, D.J., Sarris, G., Santoro, S.A., and Iozzo, R.V. (2000) Decorin binds near the C terminus of type I collagen. *J. Biol. Chem.*, **275**, 21801–21804.

Kobe, B. and Deisenhofer, J. (1994) The leucine-rich repeat: a versatile binding motif. *Trends Biochem. Sci.*, **19**, 415–421.

Kresse, H. and E. Schönherr (2001) Proteoglycans of the extracellular matrix and growth control. *J. Cell Physiol.*, **189**, 266–274

Krusius, T. and Ruoslahti, E. (1986) Primary structure of an extracellular matrix proteoglycan core protein deduced from cloned cDNA. *Proc. Natl Acad. Sci. USA*, **83**, 7683–7687.

Landgren, C., Beier, D.R., Fassler, R., Heinegärd, D., and Sommarin, Y. (1998) The mouse chondroadherin gene: characterization and chromosomal localization. *Genomics*, **47**, 84–91.

Liu, C.Y., Shiraishi, A., Kao, C.W., Converse, R.L., Funderburgh, J.L., Corpuz, L.M., Conrad, G.W., and Kao, W.W. (1998) The cloning of mouse keratocan cDNA and genomic DNA and the characterization of its expression during eye development. *J. Biol. Chem.*, **273**, 22584–22588.

Lorenzo, P., Aspberg, A., Onnerfjord, P., Bayliss, M.T., Neame, P.J., and Heinegärd, D. (2001) Identification and characterization of asporin. a novel member of the leucine-rich repeat protein family closely related to decorin and biglycan. *J. Biol. Chem.*, **276**, 12201–12211.

Mao, J.R. and Bristow, J. (2001) The Ehlers-Danlos syndrome: on beyond collagens. *J. Clin. Invest.*, **107**, 1063–1069.

Markmann, A., Hausser, H., Schönherr, E., and Kresse, H. (2000) Influence of decorin expression on transforming growth factor-beta-mediated collagen gel retraction and biglycan induction. *Matrix Biol.*, **19**, 631–636.

Maurice, D. (1957) The structure and transparency of the cornea. *J. Physiol.*, **136**, 268–286.

Neame, P.J., Sommarin, Y., Boynton, R.E., and Heinegärd, D. (1994) The structure of a 38-kDa leucine-rich protein (chondroadherin) isolated from bovine cartilage. *J. Biol. Chem.*, **269**, 21547–21554.

Oldberg, A., antonsonn, P., Lindblom, K., and Heinegärd, D. (1989) A collagen-binding 59-kd protein is structurally related to the small proteoglycans PG-S1 and PG-S2. *EMBO J.*, **8**, 2601–2604.

Pellegata, N.S., Dieguez-Lucena, J.L., Joensuu, T., Lau, S., Montgomery, K.T., Krahe, R., Kivela, T., Kucherlapati, R., Forsius, H., and de la Chapelle, A. (2000) Mutations in KERA, encoding keratocan, cause cornea plana. *Nature Genet.*, **25**, 91–95.

Piek, E., Heldin, C.H., and Ten Dijke, P. (1999) Specificity, diversity, and regulation in TGF-beta superfamily signaling. *FASEB J.*, **13**, 2105–2124.

Pusch, C.M., Zeitz, C., Brandau, O., Pesch, K., Achatz, H., Feil, S., Scharfe, C., Maurer, J., Jacobi, F.K., Pinckers, A., and others. (2000) The complete form of X-linked congenital stationary night blindness is caused by mutations in a gene encoding a leucine-rich repeat protein. *Nature Genet.*, **26**, 324–327.

Quantock, A.J., Meek, K.M., and Chakravarti, S. (2001) An x-ray diffraction investigation of corneal structure in lumican-deficient mice. *Invest. Ophthalmol. Vis. Sci.*, **42**, 1750–1756.

Quentin, E., Gladen, A., Roden, L., and Kresse, H. (1990) A genetic defect in the biosynthesis of dermatan sulfate proteoglycan: galactosyltransferase I deficiency in fibroblasts from a patient with a progeroid syndrome. *Proc. Natl Acad. Sci. USA*, **87**, 1342–1346.

Rafii, M., Creely, H., McKechnie, B., Ferri, R., Seo, N., Young, M., McQuillan, D., and Fallon, J.R. (2000) Interactions of the protoeglycan biglycan with the distrophin-associated protein complex and its roles in muscular distrophy and synaptogenesis. *Mol. Biol. Cell*, **11**(suppl), 146a.

Reardon, A.J., Le Goff, M., Briggs, M.D., McLeod, D., Sheehan, J.K., Thornton, D.J., and Bishop, P.N. (2000) Identification in vitreous and molecular cloning of opticin, a novel member of the family of leucine-rich repeat proteins of the extracellular matrix. *J. Biol. Chem.*, **275**, 2123–2129.

Säämanen, A.-M., Salminen, H.J., Rantakokko, A.J., Heinegärd, D., and Vuorio, E.I. (2001) Murine fibromodulin: cDNA and genomic structure, and age-related expression and distribution in the knee joint. *Biochem. J.*, **355**, 577–585.

Santra, M., Mann, D.M., Mercer, E.W., Skorski, T., Calabretta, B., and Iozzo, R.V. (1997) Ectopic expression of decorin protein core causes a generalized growth suppression in neoplastic cells of various histogenetic origin and requires endogenous p21, an inhibitor of cyclin-dependent kinases. *J. Clin. Invest.*, **100**, 149–157.

Schaefer, L., Macakova, K. Raslik, I., Micegova, M., Girone, H.J., Schönherr, E., Robenek, H., Echtermeyer, F.G., Grässel, S., Bruckner, P., and others. (2002) Absence of decorin adversely influences tubulointerstitial fibrosis of the obstructed kidney by enhanced apoptosis and increased inflammatory reaction. *Am. J. Pathol.*, **160**, 1181–1191.

Scholzen,T., Solursh, M., Suzuki, S., Reiter, R., Morgan, J., Buchberg, A., Siracusa, L., and Iozzo, R. (1994) The murine decorin. Complete cDNA cloning, genomic organization, chromosomal assignment, and expression during organogenesis and tissue differentiation. *J. Biol. Chem.*, **269**, 28270–28281.

Schönherr, E., Levkau, B., Schaefer, L., Kresse, H., and Walsh, K. (2001) Decorin-mediated signal transduction in endothelial cells. Involvement of Akt/protein kinase B in up-regulation of p21(WAF1/CIP1) but not p27(KIP1). *J. Biol. Chem.*, **276**, 40687–40692.

Schönherr, E., Broszat, M., Brandan, E., Bruckner, P., and Kresse, H. (1998) Decorin core protein fragment Leu155-Val260 interacts with TGF-beta but does not compete for decorin binding to type I collagen. *Arch. Biochem. Biophys.*, **355**, 241–248.

Schönherr, E., Hausser, H., Beavan, L., and Kresse, H. (1995a) Decorin-type I collagen interaction. *J. Biol. Chem.*, **270**, 8877–8883.

Schönherr, E., Witsch-Prehm, P., Harrach, B., Robenek, H., Rauterberg, J., and Kresse, H. (1995b) Interaction of biglycan with type I collagen. *J. Biol. Chem.*, **270**, 2776–2783.

Scott, I.C., Imamura, Y., Pappano, W.N., Troedel, J.M., Recklies, A.D., Roughley, P.J., and Greenspan, D.S. (2000) Bone morphogenetic protein-1 processes probiglycan. *J. Biol. Chem.*, **275**, 30504–30511.

Scott, J. (1996) Proteodermatan and proteokeratan sulfate (decorin, lumican/fibromodulin) proteins are horseshoe shaped. Implications for their interaction with collagen. *Biochemistry*, **35**, 8795–9799.

Sommarin, Y., Wendel, M., Shen, Z., Hellman, U., and Heinegärd, D. (1998) Osteoadherin, a cell-binding keratan sulfate proteoglycan in bone, belongs to the family of leucine-rich repeat proteins of the extracellular matrix. *J. Biol. Chem.*, **273**, 16723–16729.

Steere, A.C. (2001) Lyme disease. *N. Engl. J. Med.*, **345**, 115–125.

Svensson, L., Heinegärd, D., and Oldberg, Å. (1995) Decorin-binding sites for collagen type I are mainly located in leucine-rich repeats 4–5. *J. Biol. Chem.*, **270**, 20712–20716.

Svensson, L., Aszodi, A., Reinholt, F., Fassler, R., Heinegärd, D., and Oldberg, A. (1999) Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. *J. Biol. Chem.*, **274**, 9636–9647.

Svensson, L., Narlid, I., and Oldberg, A. (2000) Fibromodulin and lumican bind to the same region on collagen type I fibrils. *FEBS Lett.*, **470**, 178–182.

Sztrolovics, R., Chen, X.N., Grover, J., Roughley, P.J., and Korenberg, J.R. (1994) Localization of the human fibromodulin gene (FMOD) to chromosome 1q32 and completion of the cDNA sequence. *Genomics*, **23**, 715–717.

Takanosu, M., Boyd, T.C., Le Goff, M., Henry, S.P., Zhang, Y., Bishop, P.N., and Mayne, R. (2001) *Structure*, chromosomal location, and tissue-specific expression of the mouse opticin gene. *Invest. Ophthalmol. Vis. Sci.*, **42**, 2202–2210.

Tasheva, E.S., Corpuz, L.M., Funderburgh, J.L., and Conrad, G.W. (1997) Differential splicing and alternative polyadenylation generate multiple mimecan mRNA transcripts. *J. Biol. Chem.*, **272**, 32551–32556.

Tasheva, E.S., Funderburgh, J.L., Corpuz, L.M., and Conrad, G.W. (1998) Cloning, characterization and tissue-specific expression of the gene encoding bovine keratocan, a corneal keratan sulfate proteoglycan. *Gene*, **218**, 63–68.

Tasheva, E.S., Funderburgh, J.L., Funderburgh, M.L., Corpuz, L.M., and Conrad, G.W. (1999) Structure and sequence of the gene encoding human keratocan. *DNA Seq.*, **10**, 67–74.

Tasheva, E.S., Pettenati, M., Von Kap-Her, C., and Conrad, G.W. (2000a) Assignment of keratocan gene (KERA) to human chromosome band 12q22 by *in situ* hybridization. *Cytogenet. Cell Genet.*, **88**, 244–245.

Tasheva, E.S., Pettenati, M., Von Kap-Her, C., and Conrad, G.W. (2000b) Assignment of mimecan gene (OGN) to human chromosome band 9q22 by *in situ* hybridization. *Cytogenet. Cell Genet.*, **88**, 326–327.

Ujita, M., Shinomura, T., and Kimata, K. (1995) Molecular cloning of the mouse osteoglycin-encoding gene. *Gene*, **158**, 237–240.

Vetter, U., Vogel, W., Just, W., Young, M.F., and Fisher, L.W. (1993) Human decorin gene: intron-exon junctions and chromosomal localization. *Genomics*, **15**, 161–168.

Weber, I., Harrison, R., and Iozzo, R. (1996) Model structure of decorin and implications for collagen fibrillogenesis. *J. Biol. Chem.*, **271**, 31767–31770.

Wegrowski, Y., Pillarisetti, J., Danielson, K.G., Suzuki, S., and Iozzo, R.V. (1995) The murine biglycan: complete cDNA cloning, genomic organization, promotor function, and expression. *Genomics*, **30**, 8–17.

Whinna, H.C., Choi, H.U., Rosenberg, L.C., and Church, F.C. (1993) Interaction of heparin cofactor II with biglycan and decorin. *J. Biol. Chem.*, **268**, 3920–3924.

Wiberg, C., Hedbom, E., Khairullina, A., Lamande, S.R., Oldberg, A., Timpl, R., Morgelin, M., and Heinegärd, D. (2001) Biglycan and decorin bind close to the n-terminal region of the collagen VI triple helix. *J. Biol. Chem.*, **276**, 18947–18952.

Witsch-Prehm, H.E.P., Harrach, B., Robenek, H., Rauterberg, J., and Kresse, H. (1995) Interaction of biglycan with type I collagen. *J. Biol. Chem.*, **270**, 2776–2783.

Xaus, J., Comalada, M., Cardo, M., Valledor, A.F., and Celada, A. (2001) Decorin inhibits macrophage colony-stimulating factor proliferation of macrophages and enhances cell survival through induction of p27(Kip1) and p21(Waf1). *Blood*, **98**, 2124–2133.

Xu, T., Bianco, P., Fisher, L., Longenecker, G., Smith, E., Goldstein, S., Bonadio, J., Boskey, A., Heegaard, A., Sommer, B., and others. (1998) Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. *Nature Genet.*, **20**, 78–82.

Yamada, S., Murakami, S., Matoba, R., Ozawa, Y., Yokokoji, T., Nakahira, Y., Ikezawa, K., Takayama, S., Matsubara, K., and Okada, H. (2001) Expression profile of active genes in human periodontal ligament and isolation of PLAP-1, a novel SLRP family gene. *Gene*, **275**, 279–286.

Ying, S., Shiraishi, A., Kao, C.W.-C., Converse, R.L., Funderburgh, J.L., Swiergel, J., Roth, M.R., Comrad, G.W., and Kao, W.W.-Y. (1997) Characterization and expression of the mouse lumican gene. *J. Biol. Chem.*, **272**, 30306–30313.

Young, M.F. and Chen, X.D. (2002). Biglycan. In: Creighton, T. (ed.), *Wiley encyclopedia of molecular medicine*. Wiley, New York, pp. 363–364.